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(57) Abstract

The present invention relates to a human MIF-3 and DNA (RNA) encoding such polypeptide. Also provided is a procedure for producing such polypeptide by recombinant techniques and antibodies and antagonists/inhibitors against such polypeptide. Also provided are methods of using the polypeptide therapeutically for treating cancer, infections, acceleration of wound healing, stimulating the immune system, as an anti-inflammatory. Methods of using antibodies and antagonists/inhibitors for therapeutic purposes, is also disclosed, for example, for treating lethal endotoxaemia, ocular inflammation and diagnosing immune diseases.

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MACROPHAGE MIGRATION INHIBITORY FACTOR-3

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptide of the present invention is Macrophage Migration Inhibitory Factor-3 (MIF-3). The invention also relates to inhibiting the action of such polypeptides.

In response to antigenic or mitogenic stimulation, lymphocytes secrete protein mediators called lymphokines that play an important role in immunoregulation, inflammation and effector mechanisms of cellular immunity, (Miyajima, A., et al., FASEB J., 38:2462-2473 (1988)). The first reported lymphokine activity was observed in culture supernatants of antigenically sensitized and activated guinea pig lymphocytes. This activity was named migration inhibitory factor (MIF) for its ability to prevent the migration of guinea pig macrophages out of capillary tubes *in vitro*, (Bloom, B.R., et al., Science, 153:80-82 (1966)).

The detection of MIF activity is correlated with a variety of inflammatory responses including delayed hypersensitivity and cellular immunity (Rocklin, R.E. et al., New Engl. J. Med., 282:1340-1343 (1970); allograft rejection

(Al-Askari, S. et al., Nature, 205:916-917 (1965); and rheumatoid polyarthritic synovialis (Odink et al., Nature, 330:80-82 (1987).

MIF is a lymphokine known to be produced by activated T cells. MIF is a major secreted protein released by the anterior pituitary cells. A large number of publications have reported the isolation and identification of putative MIF molecules. For example, MIF-1 was purified to homogeneity from the serum-free culture supernatant of a human T cell hybridoma clone called F5. Oki, S., Lymphokine Cytokine Res., 10:273-80 (1991). Also, an MIF-2, which is more hydrophobic than MIF-1, was purified to homogeneity from the same clone, (Hirose, S., et al., M, Microbiol. Immunol., 35:235-45 (1991)). The polypeptide of the present invention, MIF-3, is structurally related to the MIF family.

In accordance with one aspect of the present invention, there is provided a novel mature polypeptide which is MIF-3, as well as fragments, analogs and derivatives thereof. The polypeptide of the present invention is of human origin.

In accordance with another aspect of the present invention, there are provided polynucleotides (DNA or RNA) which encode such polypeptides.

In accordance with yet a further aspect of the present invention, there is provided a process for producing such polypeptides by recombinant techniques.

In accordance with another aspect of the present invention there are provided antibodies against such polypeptides.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptides, or polynucleotides encoding such polypeptides for therapeutic purposes, for example, for treating cancer, infections, accelerating wound healing and stimulating the immune system.

In accordance with yet another aspect of the present invention, there are provided antagonist/inhibitors to such polypeptides, which may be used to inhibit the action of such polypeptides, for example, in the treatment of septic shock, lethal endotoxaemia and ocular inflammation.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figure 1 depicts the polynucleotide sequence and corresponding deduced amino acid sequence of MIF-3. The polypeptide encoded by the amino sequence shown is the preprotien form of the polypeptide, and the standard one letter abbreviation for amino acids is used.

In accordance with an aspect of the present invention, there is provided an isolated nucleic acid (polynucleotide) which encodes for the mature polypeptide having the deduced amino acid sequence of Figure 1 or for the mature polypeptide encoded by the cDNA of the clone deposited as ATCC Deposit No. 75712 on March 18, 1994.

The polynucleotide of this invention was discovered from a cDNA library derived from human T cells. It is structurally related to the human MIF family. It contains an open reading frame encoding a protein of approximately 118 amino acid residues. The protein exhibits the highest degree of homology to human MIF with 34 % identity and 78 % similarity over the entire amino acid sequence.

The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptide may be

identical to the coding sequence shown in Figure 1 or that of the deposited clone or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same, mature polypeptide as the DNA of Figure 1 or the deposited cDNA.

The polynucleotide which encodes for the mature polypeptide of Figure 1 or for the mature polypeptide encoded by the deposited cDNA may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence such as a leader or secretory sequence or a proprotein sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of Figure 1 or the polypeptide encoded by the cDNA of the deposited clone. The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same mature polypeptide as shown in Figure 1 or the same mature polypeptide encoded by the cDNA of the deposited clone as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptide of Figure 1 or the polypeptide encoded by the cDNA of the deposited clone. Such nucleotide variants

include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotide may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in Figure 1 or of the coding sequence of the deposited clone. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pQE-9 vector to provide for p· ·ification of the mature polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 50% and preferably 70% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides . As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which retain substantially the same biological function or activity as the mature

polypeptide encoded by the cDNA of Figure 1 or the deposited cDNA.

The deposit(s) referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

The present invention further relates to a MIF-3 polypeptide which has the deduced amino acid sequence of Figure 1 or which has the amino acid sequence encoded by the deposited cDNA, as well as fragments, analogs and derivatives of such polypeptide.

The terms "fragment," "derivative" and "analog" when referring to the polypeptide of Figure 1 or that encoded by the deposited cDNA, means a polypeptide which retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

The fragment, derivative or analog of the polypeptide of Figure 1 or that encoded by the deposited cDNA may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such

substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the

form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the MIF genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing polypeptides by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator.

The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila and Sf9; animal cells such as CHO, COS or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG,

pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are PKK232-8 and PCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation. (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook,

et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or

more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175

(1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The MIF-3 polypeptide can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography hydroxylapatite chromatography and lectin chromatography. It is preferred to have low concentrations (approximately 0.15-5 mM) of calcium ion present during purification. (Price et al., J. Biol. Chem., 244:917 (1969)). Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

The MIF-3 proteins of the present invetion have displayed biological activities which indicate its role as a

general activator or several different macrophage functions. Further, MIF-3 both inhbitis the migration of human macrophages and stimulates the activity of macrophages.

The MIF-3 polypeptides of the present invention may be employed as an anti-tumor agent. Activated macrophages alone or in combination with specific anti-tumor monoclonal antibodies have considerable tumoricidal capacity. Similarly, the ability of MIF-3 to promote macrophage-mediated killing of certain pathogens indicates the use of this molecule in treating various infections, including tuberculosis, Hunsen disease and Candida.

In addition, the ability of MIF-3 to prevent the migration of macrophages may be exploited in a therapeutic agent for treating wounds. Local application of MIF-3 at the site of injury may result in increased numbers of activated macrophages concentrated within the wound, thereby increasing the rate of healing of the wound.

In addition, MIF-3 may be used as a general immune stimulus to increase the immunity generated against specific vaccines. MIF proteins have the ability to enhance macrophages to present antigens to T cells. Therefore, MIF-3 may be emplyed to potentiate the immune response to different antigens. This is extremely important in cases such as AIDS or AIDS related complex.

MIF-3 may also be employed to enhance the detoxification function of the liver. There is evidence that a protein having MIF activity in the rat liver links the chemical and immunological detoxification systems. This protein actuates both glutothione S-transferase (GSTs) and MIF activity. Primary structure comparisons reveal significant similarity between GSTs and MIF.

The MIF polypeptides may also be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as "gene therapy."

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by, for example, procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells in vivo after combination with a suitable delivery vehicle.

The polypeptides of the present invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the polypeptide, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating

the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

The pharmaceutical compositions may be administered in a convenient manner such as by the topical, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. The amounts and dosage regimens of MIF and administered to a subject will depend on a number of factors such as the mode of administration, the nature of the condition being treated and the judgment of the prescribing physician. Generally speaking, they are given, for example, in therapeutically effective doses of at least about 10 μg/kg body weight and in most cases they will be administered in an amount not in excess of about 8 mg/Kg body weight per day and preferably the dosage is from about 10 μg/kg to about 1 mg/kg body weight daily, taking into account the routes of administration, symptoms, etc.

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the cDNA is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These

primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clones to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 500 or 600 bases; however, clones larger than 2,000 bp have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. FISH requires use of the clones from which the EST was derived, and the longer the better. For example, 2,000 bp is good, 4,000 is better, and more than 4,000 is probably not necessary to get good results a reasonable percentage of the time. For a review of this technique, see Verma et al., Human Chromosomes: a Manual of Basic Techniques, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same

chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that cDNA sequence. Ultimately, complete sequencing of genes from several individuals is required to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal,

preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention.

This invention if further related to a method for identification of MIF-3 receptors. The gene encoding a receptor can be identified by expression cloning, which comprises preparing polyadenylated RNA from a cell responsive to MIF-3 and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to MIF-3. Transfected cells which are grown on glass slides are exposed to labeled MIF-3. MIF-3 can be labeled by a variety of means including iodidation or inclusion of a recognition site for a site-specific protein kinase. Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and retransfected using an iterative sub-pooling and rescreening process, eventually yielding a single clone that encodes the putative receptor. As an alternative approach for receptor

identification, labeled MIF-3 can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE and exposed to x-ray film. The labeled complex containing the ligand-receptor can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of generate oligonucleotide probes to screen a cDNA library to identify the gene encoding the putative receptor.

This invention also provides a method of screening drugs to identify those which enhance (agonists) or block (antagonists) interaction of MIF-3 to its receptor. An agonist is a compound which increases the natural biological functions of MIF-3, while an antagonist eliminates such functions. As an example, a mammalian cell or membrane preparation expressing an MIF-3 receptor would be incubated with labeled MIF-3 in the presence of drug. The ability of drug to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of ligand and receptor would be measured compared in the presence or absence of drug. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

The present invention is also directed to antagonist/inhibitor molecules of the polypeptides of the present invention and their use to inhibit or eliminate the function of the polypeptide.

An example of an antagonist is an antibody against the MIF-3 polypeptide or, in some cases, an oligonucleotide which binds to the polypeptide. An antagonist may also be be a peptide derivate of MIF-3 which recognizes and binds to MIF-3 receptor sites but has no biological function thereby effectivfely blocking the receptors.

An example of an inhibitor is an antisense construct prepared using antisense technology. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the mature polypeptides of the present invention, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res., 6:3073 (1979); Cooney et al, Science, 241:456 (1988); and Dervan et al., Science, 251: 1360 (1991)), thereby preventing transcription and the production of MIF-3. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into the MIF-3 (antisense - Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of MIF-3.

The antagonist/inhibitors may be employed to protect against lethal endotoxaemia and septic shock. Cytokines, including Macrophage Migration Inhibitory Proteins, are critical in the often fatal cascade of events that causes septic shock. An endotoxin is a lipopolysaccharide (LPS) moiety of gram-negative bacillary cell walls. This endotoxin causes vaso-constriction of small arteries and veins, which leads to increased peripheral vascular resistance and decreased cardiac output. These are the symptoms of lethal endotoxaemia which leads to septic shock. Anterior pituitary cells specifically release MIF proteins in response to the presence of LPS. These pituitary-derived MIF proteins

contribute to circulating MIF proteins which are already present in the post-acute phase of endotoxaemia.

The antagonist/inhibitors may also be used to treat ocular inflammations since partial sequencing of small lens proteins has identified an MIF protein in the calf lens. Accordingly, MIF proteins may act as intercellular messengers or part of the machinery of cellular differentiation, whereby over-expression of MIF proteins may lead to ocular inflammation.

The antibodies to MIF-3 may be employed for diagnosing disease progression and efficacy of therapeutic intervention since the level of MIF-3 in circulation may correlate with the state of a disease. The antagonist/inhibitors may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinabove described.

The present invention also relates to an assay for identifying potential antagonist/inhibitors specific to MIF-3. An example of such an assay combines MIF-3 and a potential antagonist/inhibitor with membrane-bound MIF-3 receptors or recombinant MIF-3-receptors under appropriate conditions for a competitive inhibition assay. MIF-3 can be labeled, such as by radioactivity, such that the number of MIF-3 molecules bound to the receptor can determine the effectiveness of the potential antagonist/inhibitor.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available,

publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., Virology, 52:456-457 (1973).

Example 1

Bacterial Expression and Purification of MIF-3

The DNA sequence encoding for MIF-3 ATCC # 75712 is initially amplified using PCR oligonucleotide primers corresponding to the 5' terminus and sequences of the processed MIF-3 protein and the vector sequences 3' to the MIF-3 gene. Additional nucleotides corresponding to MIF-3 were added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence CCCGCATGCCGTTCCTGGAGCTGG contains an Sph I restriction enzyme site and 19 nucleotides of MIF-3 coding sequence starting from the initiation codon. The 3' sequence CCCAGATCTTAAAAAAGTCATGACCGT contains complementary sequences to a Bgl II site and is followed by 18 nucleotides preceeding the termination codon of MIF-3. The restriction enzyme sites correspond to the Sph I and Bam HI restriction enzyme sites on the bacterial expression vector pQE-70 (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). pQE-70 encodes antibiotic resistance (Amp'), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), and puts the His tag to the 3' end of the gene. pQE-70 was then digested with Sph I and Bam HI. The amplified sequences were ligated into pQE-70 and were inserted in frame with the sequence encoding for the histidine tag. Figure 2 shows a schematic representation of

this arrangement. The ligation mixture was then used to transform E. coli strain M15/rep 4 available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies were selected. Plasmid DNA was isolated and confirmed by restriction analysis. Clones containing the desired constructs were grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells were grown to an optical density 600 (O.D.^{600}) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") was then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells were grown an extra 3 to 4 hours. Cells were then harvested by centrifugation. The cell pellet was solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized MIF-3 was purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag. Hochuli, E. et al., J. Chromatography 411:177-184 (1984). MIF-3 (95 % pure) was eluted from the column in 6 molar guanidine HCl pH 5.0.

Protein renaturation out of GnHCl can be accomplished by several protocols. (Jaenicke, R. and Rudolph, R., Protein Structure - A Practical Approach, IRL Press, New York (1990)). Initially, step dialysis is utilized to remove the GnHCL. Alternatively, the purified protein isolated from the Ni-chelate column can be bound to a second column over which a decreasing linear GnHCL gradient is run. The protein is

allowed to renature while bound to the column and is subsequently eluted with a buffer containing 250 mM Imidazole, 150 mM NaCl, 25 mM Tris-HCl pH 7.5 and 10% Glycerol. Finally, soluble protein is dialyzed against a storage buffer containing 5 mM Ammonium Bicarbonate. The purified protein was analyzed by SDS-PAGE. See Figure 3.

Example 2

Expression of Recombinant MIF-3 in COS cells

The expression of plasmid, MIF-3 HA is derived from a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CMV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. A DNA fragment encoding the entire MIF-3 precursor and a HA tag fused in frame to its 3' end is cloned into the polylinker region of the vector, therefore, the recombinant protein expression is directed under the CMV promoter. The HA tag correspond to an epitope derived from the influenza hemagglutinin protein as previously described (I. Wilson, H. Niman, R. Heighten, A Cherenson, M. Connolly, and R. Lerner, 1984, Cell 37, 767). The infusion of HA tag to the target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is described as follows:

The DNA sequence encoding for MIF-3, ATCC # 75712, is constructed by PCR on the original EST cloned using two primers: the 5' primer CCCAAGCTTATGCCGTTCCTGGAACTG contains a Hind III site followed by 18 nucleotides of MIF-3 coding sequence starting from the initiation codon; the 3' sequence CGCTCTAGATCAAGCGTAGTCTGGGACGTCGTATGGGTATAAAAAAGTCATGACCGTC contains complementary sequences to an Xba I site, translation stop codon, HA tag and the last 19 nucleotides of

the MIF-3 coding sequence (not including the stop codon). Therefore, the PCR product contains a Hind III site, MIF-3 coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag, and an Xba I site. The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with Hind III and Xba I restriction enzymes and ligated. The ligation mixture is transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture is plated on ampicillin media plates and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant MIF-3, COS cells are transfected with the expression vector by DEAE-DEXTRAN method. (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the MIF-3 HA protein is detected by radiolabelling and immunoprecipitation method. (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells are labelled for 8 hours with ^{35}S-cysteine two days post transfection. Culture media are then collected and cells are lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5). (Wilson, I. et al., Id. 37:767 (1984)). Both cell lysate and culture media are precipitated with a HA specific monoclonal antibody. Proteins precipitated are analyzed on 15% SDS-PAGE gels.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: , ET AL.

(ii) TITLE OF INVENTION: Macrophage Migration Inhibitory Factor - 3

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: CARELLA, BYRNE, BAIN, GILFILLAN, CECCHI, STEWART & OLSTEIN
(B) STREET: 6 BECKER FARM ROAD
(C) CITY: ROSELAND
(D) STATE: NEW JERSEY
(E) COUNTRY: USA
(F) ZIP: 07068

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 INCH DISKETTE
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: MS-DOS
(D) SOFTWARE: WORD PERFECT 5.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: FERRARO, GREGORY D.
(B) REGISTRATION NUMBER: 36,134
(C) REFERENCE/DOCKET NUMBER: 325800-115

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 201-994-1700
(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 357 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATGCCGTTCC TGGAGCTGGA CACGAATTTG CCCGCCAACC GAGTGCCCGC GGGGCTGGAG  60
AAACGACTCT GCGCCGCCGC TGCCTCCATC CTGGGCAAAC CTGCGGACCG CGTGAACTGT 120
ACGGTACGGC CGGGCCTGGC CATGGCGCTG AGCGGGTCCA CCGAGCCCTG CGCGCAGCTG 180
TCCATCTCCT CCATCGCCGT AGTGGGCACC GCCGAGGACA ACCCCAGCCA ACGCGCCCAC 240
TTCTTTGAGT TTCTCACCAA GGAGCTAGCC CTGGGCCAGG ACCGGATACT TATCCGCTTT 300
TTCCCCTTGG AGTCCTGGCA GATTGGCAAG ATAGGGACGG TCATGACTTT TTTATGA    357
```

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 118 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Pro Pag Leu Glu Leu Asp Thr Asn Leu Pro Ala Asn Arg Val
5 10 15
Pro Ala Gly Leu Glu Lys Arg Leu Cys Ala Ala Ala Ala Ser Ile
20 25 30
Leu Gly Lys Pro Ala Asp Arg Val Asn Val Thr Val Arg Pro Gly
35 40 45
Leu Ala Met Ala Leu Ser Gly Ser Thr Glu Pro Cys Ala Gln Leu
50 55 60
Ser Ile Ser Ser Ile Gly Val Val Gly Thr Ala Glu Asp Asn Arg
65 70 75
Ser His Ser Ala His Phe Phe Glu Phe Leu Thr Lys Glu Leu Ala
80 85 90
Leu Gly Gln Asp Arg Ile Leu Ile Arg Phe Phe Pro Leu Glu Ser
95 100 105
Trp Gln Ile Gly Lys Ile Gly Thr Val Met Thr Phe Leu
110 115

WHAT IS CLAIMED IS:

1. An isolated polynucleotide selected from the groups consisting of

(a) a polynucleotide encoding an MIF-3 polypeptide having the deduced amino acid sequence of Figure 1 or a fragment, analog or derivative of said polypeptide;

(b) a polynucleotide encoding an MIF-3 polypeptide having the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 75712 or a fragment, analog or derivative of said polypeptide.

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 1 wherein the polynucleotide is genomic DNA.

5. The polynucleotide of Claim 2 wherein said polynucleotide encodes MIF-3 having the deduced amino acid sequence of Figure 1.

6. The polynucleotide of Claim 2 wherein said polynucleotide encodes the MIF-3 polypeptide encoded by the cDNA of ATCC Deposit No. 75712.

7. The polynucleotide of Claim 1 having the coding sequence for MIF-3 as shown in Figure 1.

8. The polynucleotide of Claim 2 having the coding sequence for MIF-3 deposited as ATCC Deposit No. 75712.

9. A vector containing the DNA of Claim 2.

10. A host cell genetically engineered with the vector of Claim 9.

11. A process for producing a polypeptide comprising: expressing from the host cell of Claim 10 the polypeptide encoded by said DNA.

12. A process for producing cells capable of expressing a polypeptide comprising genetically engineering cells with the vector of Claim 9.

13. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having MIF-3 activity.

14. A polypeptide selected from the group consisting of (i) a MIF-3 polypeptide having the deduced amino acid sequence of Figure 1 and fragments, analogs and derivatives thereof and (ii) a MIF-3 polypeptide encoded by the cDNA of ATCC Deposit No. 75712 and fragments, analogs and derivatives of said polypeptide.

15. The polypeptide of Claim 14 wherein the polypeptide is MIF-3 having the deduced amino acid sequence of Figure 1.

16. An antibody against the polypeptide of claim 14.

17. An antagonist/inhibitor against the polypeptide of claim 14.

18. A method for the treatment of a patient having need of MIF-3 comprising: administering to the patient a therapeutically effective amount of the polypeptide of claim 14.

19. A method for the treatment of a patient having need to inhibit MIF-3 comprising: administering to the patient a therapeutically effective amount of the antagonist/inhibitor of Claim 17.

20. A pharmaceutical composition comprising the polypeptide of Claim 14 and a pharmaceutically acceptable carrier.

21. The method of Claim 18 wherein said therapeutically effective amount of the polypeptide is administered by providing to the patient DNA encoding said polypeptide and expressing said polypeptide *in vivo*.

22. A method for identifying antagonist/inhibitors to MIF-3 comprising:

combining labeled MIF-3 and a potential antagonist/inhibitor receptors for MIF-3 under appropriate conditions such that a competitive binding assay occurs;

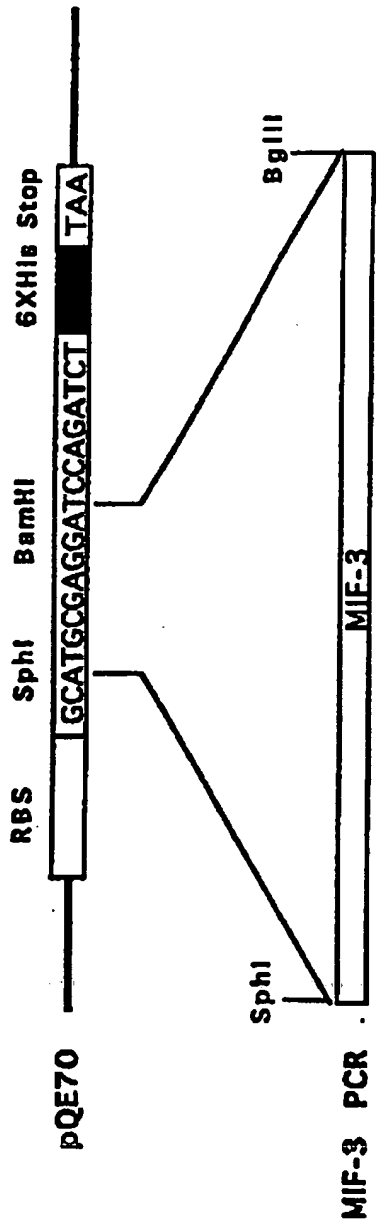
determining the amount of MIF-3 bound to the receptors; and

identifying the effectiveness of the potential antagonist/inhibitor.

FIG. 1

```
1   ATGCCGTTCCTGGAGCTGGACACGAATTTGCCGGCCAACCGAGTGCCCGCGGGGCTGGAG   60
    M  P  F  L  E  L  D  T  N  L  P  A  N  R  V  P  A  G  L  E

61  AAACGACTCTGCGCCGCCGCTGCCTCCATCCTGGGCAAACCTGCGGACCGCGTGAACGTG  120
    K  R  L  C  A  A  A  A  S  I  L  G  K  P  A  D  R  V  N  V

121 ACGGTACGGCCGGGCCTGGCCATGGCGCTGAGCGGGTCCACCGAGCCCTGCGCGCAGCTG  180
    T  V  R  P  G  L  A  M  A  L  S  G  S  T  E  P  C  A  Q  L

181 TCCATCTCCTCCATCGGCGTAGTGGGCACCGCCGAGGACAACCGCAGCCACAGCGCCCAC  240
    S  I  S  S  I  G  V  V  G  T  A  E  D  N  R  S  H  S  A  H

241 TTCTTTGAGTTTCTCACCAAGGAGCTAGCCCTGGGCCAGGACCGGATACTTATCCGCTTT  300
    F  F  E  F  L  T  K  E  L  A  L  G  Q  D  R  I  L  I  R  F

301 TTCCCCTTGGAGTCCTGGCAGATTGGCAAGATAGGGACGGTCATGACTTTTTTATGA    357
    F  P  L  E  S  W  Q  I  G  K  I  G  T  V  M  T  F  L  *
```

FIG. 2


RBS
SphI
BamHI
GCATGCGAGGATCCAGATCT
6XHis Stop
TAA
BglII
MIF-3
SphI
pQE70
MIF-3 PCR

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05385

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :536/23.5, 23.1; 435/320.1, 252.3, 69.1; 514/12; 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5, 23.1; 435/320.1, 252.3, 69.1; 514/12; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG - biotech files, GENEMBL sequence databases

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Archives of Biochemistry and Biophysics, Volume 303, Number 1, issued 15 May 1993, Zeng et al, "The Major Binding Protein of the Interferon Antagonist Sarcolectin in Human Placenta Is a macrophage Migration Inhibitory Factor", pages 74-80, see entire document.	1-15, 18, 20
A	Lymphokine and Cytokine Research, Volume 10, Number 4, issued August 1991, Oki et al, "Macrophage Migration Inhibitory Factor (MIF) Produced by a Human T Cell Hybridoma Clone", pages 273-280, see entire document.	1-15, 18, 20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
26 OCTOBER 1994

Date of mailing of the international search report
07 NOV 1994

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05385

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Electrophoresis, Volume 13, issued 1992, Hochstrasser et al, "Human liver protein map: A reference database established by microsequencing and gel comparison", pages 992-1001, see entire document.	1-15, 18, 20
A	Molecular and Cellular Biology, Volume 12, Number 9, issued September 1992, Lanahan et al, "Growth Factor-Induced Delayed Early Response Genes", pages 3919-3929, see entire document.	1-15, 18, 20
A,P	Nature, Volume 365, issued 21 October 1993, Bernhagen et al, "MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia", pages 756-759, see entire document.	1-15, 18, 20
A	Proceedings of the National Academy of Sciences USA, Volume 90, issued February 1993, Wistow et al, "A macrophage migration inhibitory factor is expressed in the differentiating cells of the eye lens", pages 1272-1275, see entire document.	1-15, 18, 20
A	Proceedings of the National Academy of Sciences USA, Volume 86, issued October 1989, Weiser et al, "Molecular cloning of a cDNA encoding a human macrophage migration inhibitory factor", pages 7522-7526, see entire document.	1-15, 18, 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05385

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-15, 18, 20

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05385

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

C07H 17/00; C12N 15/00, 1/20; C12P 21/06; A61K 37/00; C07K 3/00, 13/00, 15/00, 17/00

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-15, 18, and 20, drawn to DNA, peptides encoded thereby, host cells expressing same, methods of expressing same, and methods of administering the peptides.

II. Claim 16, drawn to an antibody.

III. Claims 17, 19, and 22, drawn to antagonists and methods of using same.

IV. Claim 21, drawn to gene therapy.

The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept. Moreover, the different inventive groups are structurally and functionally distinct, and can be used in materially different processes. For example, the peptides of Group I can be used in therapy, which is distinct from the other inventive Groups. The antibodies of Group II can be used in diagnostic methods, which is distinct from the other inventive groups. The antagonists of Invention III can be used to block MIF-3 function, which is distinct from the other inventive groups. Finally, the gene therapy of Group IV is distinct from the other inventive groups in that considerations of gene regulation and transcriptional activation factors must be considered, which is not required by any of the other inventive groups.